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FERTILIZATION AND MORTALITY RATE OF ATLANTIC SALMON (SALMO SALAR) EGGS DURING INCUBATION

by
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Salmon Genetics Research Program

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St. Andrews, N. B.

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FERTILIZATION AND MORTALITY RATE OF ATLANTIC SALMON (SALMO SALAR) EGGS DURING INCUBATION

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ABSTRACT

The relationship between the fertilization and mortality rates of Atlantic salmon eggs during incubation was investigated. Samples of eggs were preserved and cleared 24 hours after the mixing of gametes. Three categories of eggs were identified by eye and with a compound microscope: unfertilized, questionable and fertilized. Questionable embryos, although sometimes fertilized, were not developing. Fertilization rate was defined as the number of developing embryos divided by the total number in the sample, expressed as a percentage. Fertilization rate at the 4-cell stage was quite easily determined and more accurate than other current methods. Cumulative rate of egg mortality at 2, 14, 30 and 60 days was significantly negatively correlated to fertilization rate. The highest correlation observed was -.75 at 60 days, which corresponds to the eyed stage one day after shocking of the eggs. Thus, many eggs which were unfertilized or not developing did not turn white until they had been incubated more than 30 days. Fertilization

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rate was not correlated to survival to the 1g fry stage. When fertilization rate was less than 75 percent, mortality rate exceeded 50 percent after 60 days of incubation. The results of the study suggest that determination of fertilization rate is a useful tool in both hatchery and scientific settings. The findings also emphasize the role of many other factors that cause mortality during incubation.

INTRODUCTION

Fertilization rate can be used as an indicator of the success of the spawning techniques,(Gordon et al. 1987), and to account for mortality in scientific experiments (Springate et al. 1984; Beacham 1988) and hatchery operations (Piper et al. 1982). In a salmon hatchery, many diverse factors determine which eggs, of the original number stripped from the female and combined with milt, will survive to later stages. These factors include water quality, disease, mechanical shock due to handling, light exposure and gamete quality (Gordon et al. 1987). Both unfertilized eggs and developing embryos turn white when they die, due to the coagulation of the yolk proteins. Thus it is impossible to distinguish whether dead eggs have been fertilized without clearing and examining them. This study was initiated to evaluate the relative contribution of unsuccessful fertilization and early cleavage to the overall mortality during incubation.

In the aquaculture industry, "green" eggs are purchased on the basis of the numbers of apparently viable eggs 24-48 hours after the mixing of the gametes. It is assumed that egg lots with low fertilization rates will manifest this early on by turning white. One might predict that during the period of egg incubation, the correlation between fertilization rate and cumulative mortality would decrease. Other factors, mentioned above, would then increasingly influence mortality rate during the latter period of egg incubation. The relationships between fertilization rate and

cumulative egg mortality during incubation, and between fertilization rate and survival of fry were specifically of interest in the present study.

MATERIALS AND METHODS

Broodstock that were first time spawners, held in sea water at the Salmon Demonstration and Development Farm, Lime Kiln Bay, St. George, N. B. They were removed and stripped immediately on site during November of 1989. Egg lots were derived from single pair matings at the Atlantic Salmon Federation. The 'dry' method of fertilization was used. Milt was added to the eggs and the gametes were mixed by hand and held for two minutes. Fresh water was added to the mixture for two minutes, egg lots were then thoroughly rinsed, and allowed to water harden for two hours, before they were transferred to individual incubation trays. Appearance of the milt, eggs and ovarian fluid was noted.

A total of one hundred eggs were removed from each egg lot twenty-three hours after fertilization (±1 hour) at an incubation temperature of 8.5°C. Most embryos had reached the 4-cell stage at that time. Two point locations in each tray were randomly selected and 50 eggs collected from each location. No eggs were removed from the trays prior to this time. The eggs were then placed in Stockard's solution which acts to both preserve and clear them.

Initially, all eggs were examined individually with a compound microscope on low power. It was soon evident that the development of most embryos could be assessed without use of the microscope.

Eggs of irregular appearance were those in which the outline of one or more individual cells or the obvious appearance of a 4 or 8-cell stage could not be seen. These were further examined with the microscope.

White (dead) eggs were removed daily and the number recorded. When the majority of eggs had reached the eyed stage (about 2 months of incubation at 8.5°C), they were shocked. This was done by siphoning the eggs and water from the trays into a bucket. The contents were then poured, from a height of approximately 30 cm, into a second bucket containing water, and then returned to their hatchery tray. Both white and clear eggs (eggs without embryos) were removed the following day and the number of remaining eyed eggs was estimated volumetrically. All white eggs from eight randomly selected families were preserved post-shocking. Their appearance was compared to that observed for eggs 24 hours after mixing of gametes.

Cumulative mortality was determined after: 2, 14, 30 and 60 days of incubation. The eggs were shocked after 59 days of incubation. Cumulative mortality is expressed as a percentage of the number of eggs laid down.

Survival to the fry stage (approximately 1 g weight) was also determined by weight measurements and expressed as the number surviving to the fry stage divided by the number alive at shocking (the number of eyed eggs).

RESULTS

Twenty three hours after fertilization, embryos were generally in the 4 or 8-cell stage (Fig.1A,B). Two and 16-cell embryos were rarely seen. Families tended to demonstrate synchronous development. However, the development of several embryos within each family group often lagged behind the others. These were otherwise normal in appearance but apparently slower to divide - often a full stage behind or in a transition stage.

Three categories of embryos/eggs were observed: fertilized, unfertilized and 'questionable'. Embryos were considered to be fertilized and developing normally if they had the following appearance under the compound microscope: white, spherical or oval cells with well-defined and dark, opaque cell borders (Fig.1A,B). The embryo in side-view was "button-shaped" i.e. rather flat and lying on top of the spherical yolk. An increase in cell numbers generally occurred along this same plane (tangential to the yolk surface). The yolk globules were sometimes apparent beneath the thinner parts of the cytoplasm that marked the cell-divisions. Unfertilized eggs were translucent (clear) with an accumulation of orange yolk at one pole (Fig. 1C). These were obvious and easy to identify. Questionable embryos had a variety of appearances. Entire families, as well as individual embryos within families displaying normal development, tended to fall within this category. They appeared to develop abnormally. Odd shaped embryos resulted from unequal cleavage or deformities (Fig.1D,type 1). Within some

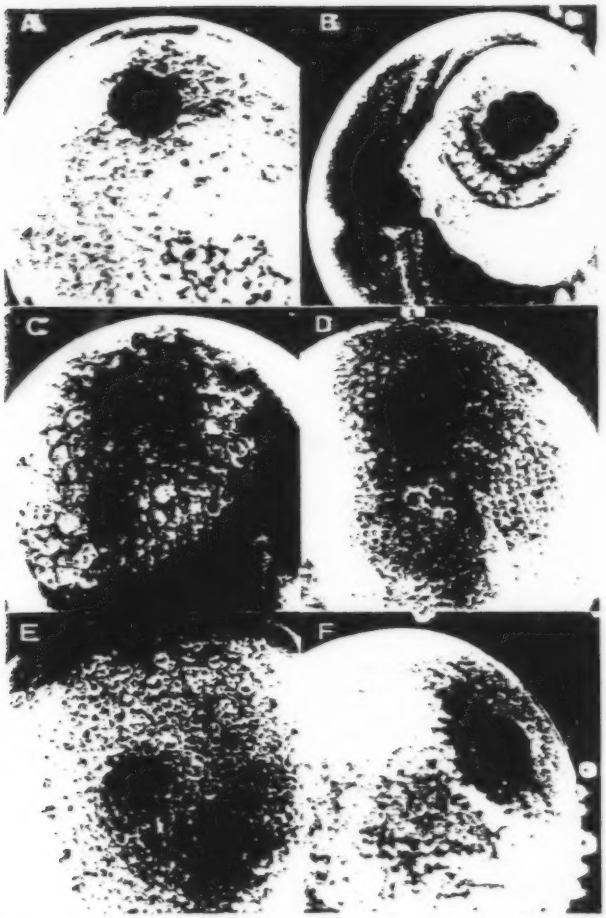


Fig. 1. Embryos and unfertilized eggs of various appearance.

A. The four cell stage.

B. The eight cell stage.

C. Unfertilized egg.

D. Fertiltized egg; embryo showing unequal cleavage of deformity.

E. Egg or embryo of rare appearance.

F. Fertilized egg; not cleaving, or undergoing retarded development.

families, embryos were characterized by a white concentration of tissue lacking well-defined edges, (Fig. 1E, type 2). The most common appearance was a white, well-defined mound or hemisphere of tissue with clearly defined borders (type 3). It protruded from the centre of the egg (Fig.1F) rather than being flatter in shape as in type 2 (Fig.1E). Although no cleavage was evident, both the shape and discreteness of type 3 and some type 1 embryos suggest that fertilization had occurred.

Families with large numbers of the various types of questionable embryos (>20 percent) had very poor survival to the eyed stage. The irregular appearance previously described suggests that development was abnormal. Questionable embryos as well as unfertilized eggs were considered not to be developing. Fertilization rate within a family group was defined as the number of embryos developing 24 hours after the mixing of gametes, divided by the total number (generally 100). According to Gordon et al. (1987) this is accurate to within ±5 percent.

The mean fertilization rate for all families was 85 ±3 percent. Figure 2 shows the cumulative mortalities to the fry stage. There was a significant negative correlation between fertilization rate and cumulative mortality at all times during egg incubation (Table 1). The highest correlation was observed at time of shocking (60 days incubation). Elevated mortality was not obvious in families with lower fertilization rates two or even 14 days after spawning (Table 1,Fig. 3). Most fertilization-related

egg mortality only became obvious after one month of incubation (Table 1, Fig. 4). Most non-developing embryos or unfertilized eggs did not turn white until they were shocked. Even after this the hatchery staff still observed transparent, uneyed eggs.

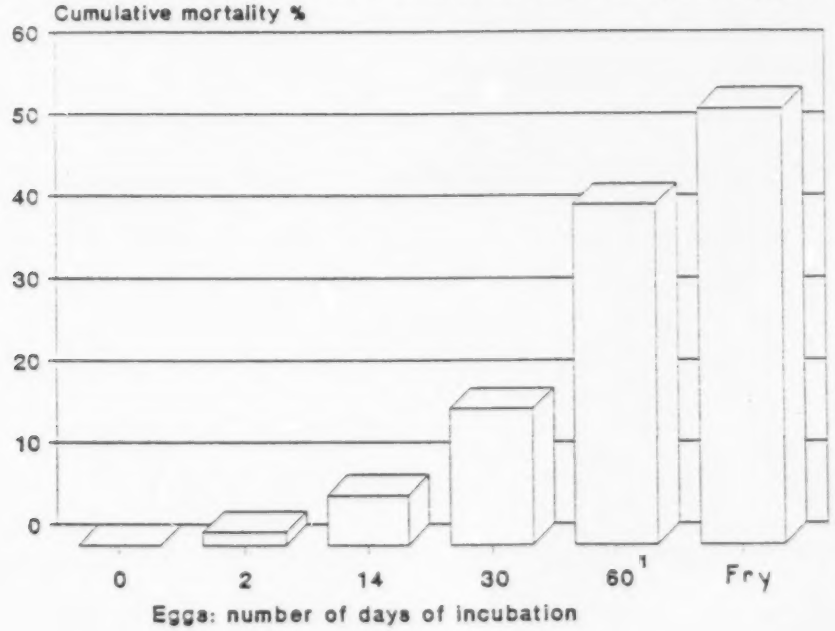


Fig. 2. Cumulative mortality[2](%) of various stages of early development.

[1] The eyed stage, one day after shocking.
[2] Total number of mortalities/number of eggs laid down X 100.

When fertilization rate was less than 60 percent, mortality at time of shocking was very high (>85 percent, Fig. 4). However, several families with high fertilization rates also had high

cumulative mortalities. There was no significant correlation between survival to the fry stage and fertilization rate (Table 1).

Table 1. Correlations between fertilization rate and cumulative mortalities after 2, 14, 30 and 60 days of incubation, and or survival as 1g fry.

		Cumulative mortalities at Days of incubation of eggs				
		2	14	30	60[1]	Survival as Fry
Fertilization Rate	R[3]	-.28	-.33	-.29	-.75	.14
	P[4]	0.011	0.003	0.009	<0.001	0.25

[1]Survival to fry = number of live fry (1g size)/number of embryos alive at eyed stage X 100%.

[2]The eyed stage, one day after shocking of eggs.

[3]Pearson's correlation coefficient.

[4]Probability of a greater absolute value of R under the null hypothesis that Rho = 0.

Urine-contaminated milt and the products of broken eggs may have affected fertilization rate in 7 of 13 families with low (<75 percent) fertilization rates. Milt was obviously yellow-coloured in five of the seven cases. The female parents of the other two families contained large numbers of egg shells from the previous spawning season and their ovarian fluid was milky.

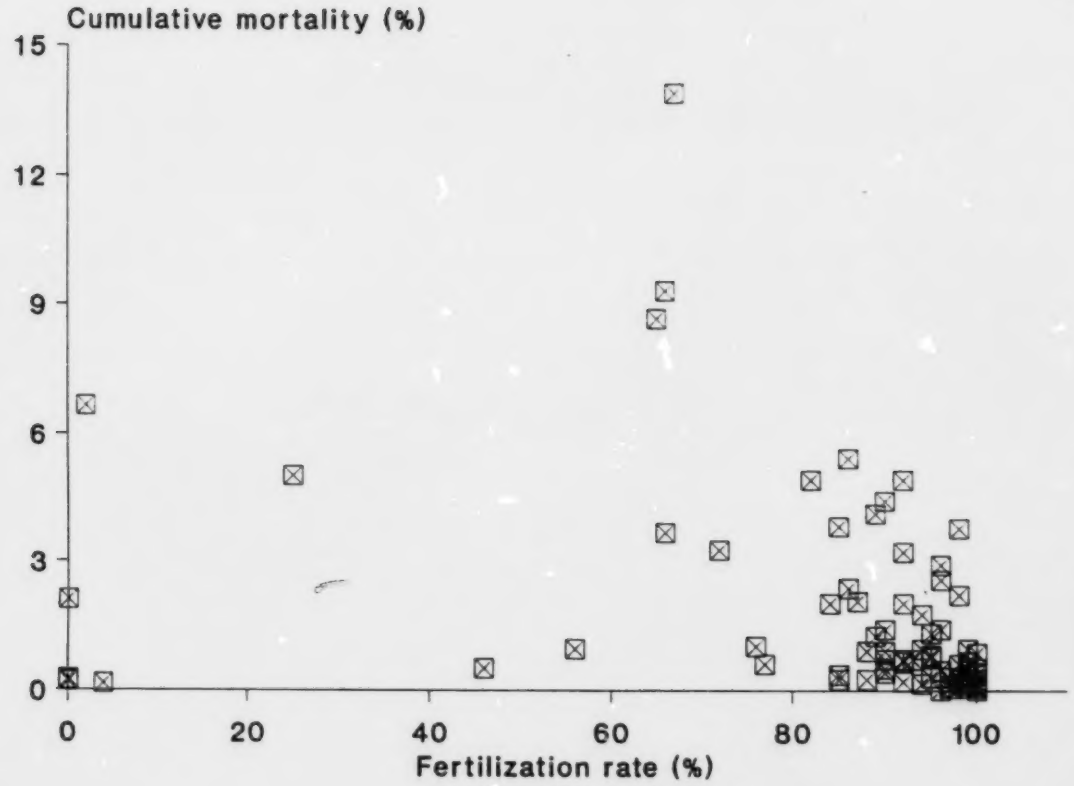


Fig. 3. Relationship between cumulative mortality at 2 days of incubation and fertilization rate.

The white eggs collected from eight families which were cleared one day after shocking revealed some larvae in advanced stages of development. However, large numbers of eggs (60 percent) in all eight families were "clear". There were no obvious embryos or tissue, with the exception of pieces of white material scattered in and between the yolk globules clustered at one pole. One egg had a tiny larva which was partially decomposed. These same

families, twenty-four hours after the mixing of gametes, had a majority of 4 or 8-cell stages or obvious type 1 and 3 embryos. These embryos had apparently decomposed by time of shocking.

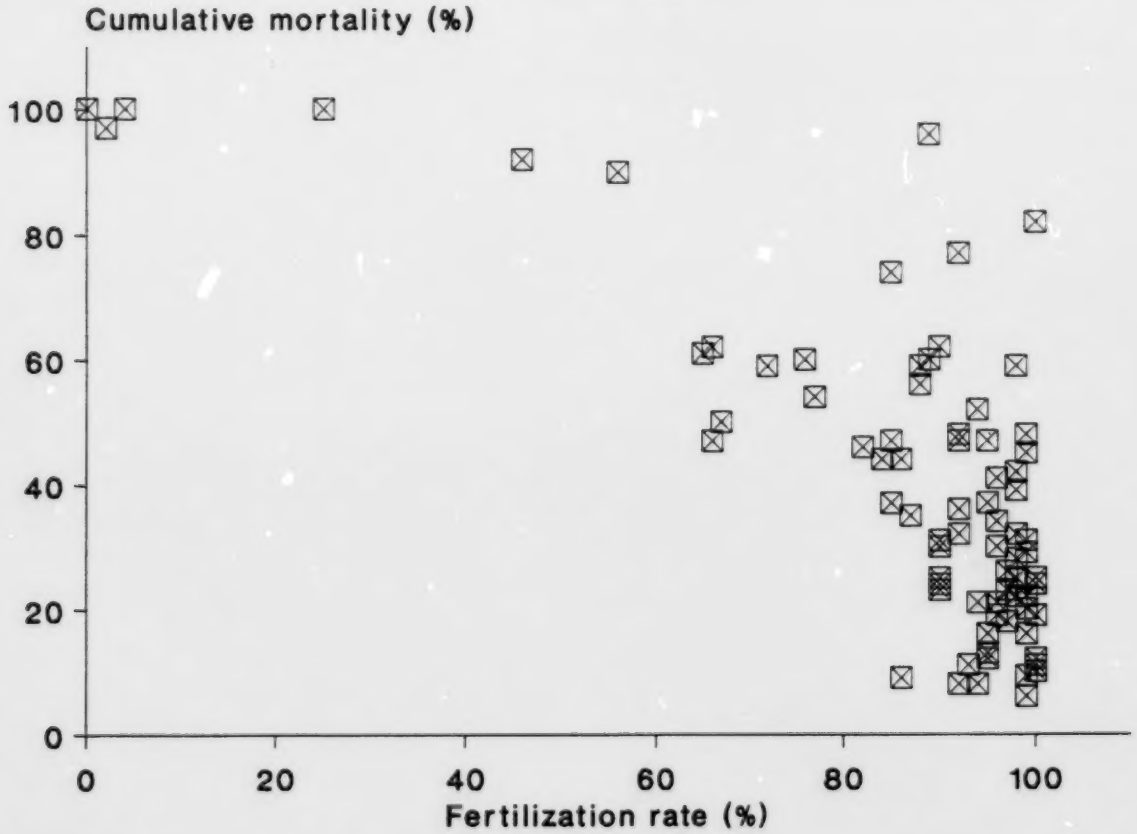


Fig. 4. Relationship between cumulative mortality at 60 days of incubation[1] and fertilization rate.

[1] The eyed stage, 1 day after shocking of eggs.

DISCUSSION

In this study, fertilization rate was determined in an attempt to identify egg mortality during incubation due to unsuccessful fertilization. It is important to distinguish between the determination of fertilization rate and the actual process of fertilization. Fertilization begins when sperm become attached to the egg and ends with the formation of a zygote having a diploid number of chromosomes (Kimball 1978). It involves the fusion of the sperm and egg nuclei. If the occurrence of fertilization were the principal concern, then some type of costly cellular or molecular technique might be desirable. In aquaculture, the principal interest is in estimating how many eggs are fertilized and consequently are developing as normal embryos.

The terms fertilization success, fertilization rate and percentage fertilization have been used widely in both the scientific literature and by hatchery operators (Gordon et al. 1987; Rosenberg 1983; Springate and Bromage 1984). In this study, the term fertilization rate was used. Although type 3 (Fig. 1F) and some other questionable embryos were apparently fertilized, they had not cleaved within 24 hours or were not developing normally. Thus, "fertilization" as used in this study, refers to the developmental events of the first 24 hours under experimental conditions. Generally, fertilization rate as determined here and in other studies would more correctly be called "early development rate". Values of fertilization rate reflect the method used to determine it. Springate and Bromage (1984), who studied rainbow

trout eggs, refer to two possible measures: the number of fertile eggs after 12 hours of incubation at 10°C (cleavage of the germinal disc is evident) or the presence of a developing embryo appearing as a white streak after seven days of incubation at 10°C.

The latter method is said to be easier to measure in terms of timing, which is not so crucial, and with respect to interpretation (Springate and Bromage 1984). The disadvantages are twofold. Mortalities for causes other than unsuccessful fertilization will have occurred within seven days. Dead eggs are picked daily at the Atlantic Salmon Federation and other hatcheries, so the ratio of fertile to infertile eggs in a given sample will have changed by the seventh day. Secondly, decisions regarding the success of spawning and the need for additional spawning are delayed. The 4-cell stage was not difficult to determine, even without the use of a microscope. Timing was more critical but there were still at least two hours in which to sample all egg lots for the 4-cell stage.

In hatchery situations (pers.obs.) and in scientific experiments (Daye and Glebe 1984), confusion in terminology may obscure meaning: fertilization success is sometimes equated with survival to the eyed stage. Using these two terms synonymously is misleading since many factors during incubation, such as husbandry practices, egg quality and water quality, determine survival to the eyed stage.

Some researchers determine fertilization rate by preserving eggs as they turn white (Beacham and Murray 1985). Examination of

the white eggs from eight families which were preserved after shocking and the partially decomposed larva suggest that degradation of embryos may occur before the eggs turn white. This would lead to a clear appearance of eggs (as in Fig. 1C) and false identification as unfertilized. Thus, clearing of white eggs later in development may not provide accurate information regarding the rate of fertilization or the stage at which death occurs.

Fertilization rates have not been determined in previous years at the Atlantic Salmon Federation. The mean survival of 82 families to the eyed stage in the Salmon Genetics Research Program was 41 percent in 1988/89. Many of these losses were probably due to poor milt quality (small quantities, often contaminated with urine and faeces) and resultant poor fertilization (Carney pers. comm.). The mean fertilization rate in 1989/90 was quite high, 85 percent, and survival to the eyed stage rose to 59 percent. Milt was generally abundant and uncontaminated. Water is known to affect sperm by prematurely activating them so that they are no longer motile when they come in contact with the ova (Gordon et al. 1987; Piper et al. 1982). Urine has a similar effect, and may have been a factor in five of the 13 families where low fertilization rates were observed. In two other families, the milky ovarian fluid and the large number of egg shells from the previous spawning season, respectively, probably contributed to a decreased fertilization rate. The milky appearance indicates broken eggs and coagulation of yolk proteins, which inhibit sperm motility (Wilcox et al. 1984).

The survival rates from the green egg stage to the eyed stage of 59 percent and to the 1g fry stage of 46 percent were comparable to other hatcheries in the region. The average survival rate of Chinook eggs from pen-reared brookstock to the eyed stage is 50 percent with a typical range of 32-87 percent (Gordon et al. 1987). Springate and Bromage (1984) note survival rates of about 45 percent for rainbow trout to the 4.5 g fry stage.

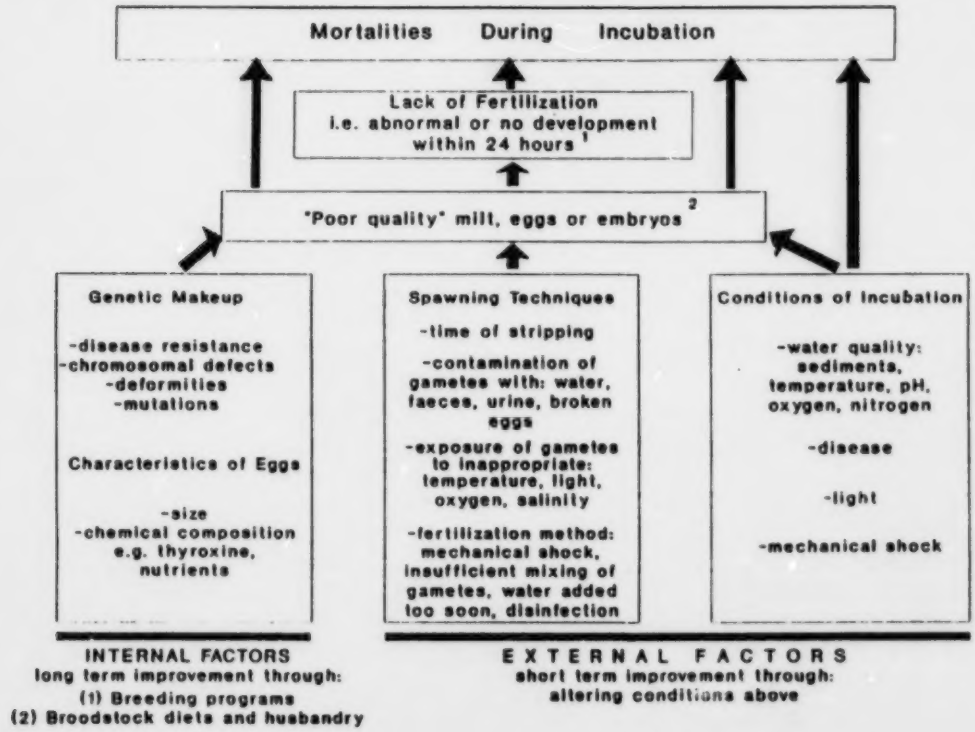
Cumulative mortality at all stages of incubation was significantly negatively correlated to fertilization rate. However, rather than decreasing with incubation time, the correlation was highest and most significant one day after shocking (Table 1). This indicates that the majority of mortalities attributable to poor fertilization appeared at time of shocking. In practical terms, if unfertilized eggs do not turn white until well after purchase, the buyer may be paying for a product of unknown quality.

Some families with high fertilization rates also experienced extensive mortality (Fig. 4). However, mean mortality to the eyed stage for families with fertilization rates of <90 percent was 28 percent, compared to the mean of 41 percent for all families (Fig. 2). Mortalities in the former were unrelated to fertilization and may have been due to problems in the hatchery such as water quality, sedimentation, fungus, inadequate oxygen and/or flow rates and mechanical shock. Broodstock holding conditions, especially salinity, water temperature (G. Farmer, pers. comm.) and diet may also play an important role in determining gamete quality and eventual survival rate.

The greatly increased mortality in families with lower fertilization rates (<75 percent) may imply some weakness. For example, a family with a 60 percent fertilization rate had experienced 90 percent mortality at shocking time. In rainbow trout, high correlations between fertilization rate and survival to eying, hatching, swimup and to the fry stage have been documented (Springate and Bromage 1984; Springate et al. 1984). Springate and Bromage (1984) note that percentage fertilization can be used as a reliable indicator of the mortalities that might be expected from a particular batch of eggs at each of the subsequent stages of development. The negative correlation of -.75 between fertilization rate and cumulative mortality to the eyed stage (60 days,Table 1), supports their findings. The lack of a significant correlation between fertilization rate and survival to the fry stage may be due to: (1) the lack of families having low fertilization rates, (mortality of these families approached 100 percent at time of shocking) and/or (2) the greater importance of other factors affecting survival of fry.

No biological explanation was offered for the high correlations reported in Springate and Bromage (1984). However, fertilization rates varied with the elapsed time between ovulation and stripping, indicating that the ripeness of the eggs affected fertilization rates (Springate et al., 1984). Chemicals in the eggs such as hormones probably play an important role both in their study and in the present one. Brown (1990) has found that thyroid hormones in yolk correlated closely with early survival of striped

bass. The factors causing mortality during incubation are therefore numerous and complex, as is the concept of gamete quality. Some of these factors are summarized in the accompanying flow chart (Fig. 5). Successful fertilization, as demonstrated by high fertilization rate, is one important step in ensuring improved survival at later stages of development in a salmon hatchery.



[1] Definition used in this study; other methods/definitions could be substituted
[2] 'Embryo' refers to eggs post-fertilization and 'eggs' to female gametes pre-fertilization

Fig. 5. Flowchart showing some of the factors resulting in mortalities during incubation.

SUMMARY AND RECOMMENDATIONS

Samples of eggs for determination of fertilization rate should be taken at a single point in time, preferably the 4-cell stage. In this study, egg lots with fertilization rates below 75 percent experienced greatly increased mortality, often not obvious because the eggs did not turn white until several weeks of incubation. A purchaser of eggs would be safer to buy eyed eggs after shocking has taken place. However, by determining fertilization rate, some of the risk of buying green eggs of unknown quality could be alleviated. A hatchery operator could readily identify and replace egg lots with poor fertilization. Hours of labour spent removing mortalities ("picking eggs") during incubation could be saved.

Determination of fertilization rates may indicate a potentially important source of mortality. Specifically, in a commercial operation, if poor fertilization or developmental problems are identified within 24 hours, other factors during incubation such as water quality or disease will not be suspected. In scientific experiments, examining fertilization rate may help to identify the effect of a certain treatment, for instance, in investigations of hybridization or triploidy.

This study serves as a reminder that many other complex factors besides poor fertilization, both environmental and genetic, result in mortality during egg and fry stages. Further studies should determine the reason(s) for the significant mortality in families where fertilization rate was high.

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